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FOREWORD

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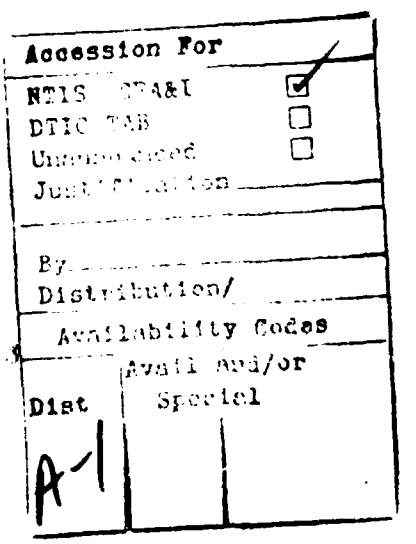

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Background

A wide variety of viruses which pose little or no public health threat in the United States are significant problems for military personnel stationed in parts of the world where these viruses are endemic. Many of these viruses are dependent on an insect vector and are found primarily in tropical regions. Infections with these viruses result in symptoms which range from temporarily debilitating to extremely serious and many are potentially fatal. The large number of these viruses and the difficulties involved in working with many of them make vaccination an impractical goal at this time. Natural or synthetic compounds which enhance the ability of the immune system to defend against these viruses would provide broader spectrum management of such infections. The prophylactic and/or therapeutic use of such agents could provide the critical difference in the outcome of situations involving large scale virus exposure of military personnel.

Generation of an immune response involves complex interactions among monocytes, antibody producing B lymphocytes, and effector and regulatory T lymphocytes, as well as a number of factors produced by these cells. Which aspects of the immune response are most important in the defense against viral infections differs for different viruses, depending on virus structure, the cell types which the virus preferentially infects, and previous exposure and immunization of the host to related viruses. A choice of a compound or combination of compounds for in vivo use to enhance antiviral immunity will therefore depend on the types of viruses likely to be encountered, history of previous exposure of the individuals involved, and whether the compounds are intended to be used prophylactically or as therapy in recently infected individuals.

It is clear that an understanding of the basic effects of given immune response modulators on individual components of the immune system is required before decisions can be made concerning which ones warrant further study. A compound may affect one or more cellular subpopulations of the immune network and the effects may be direct or indirect. Enhancement of immune responsiveness may occur by affecting cells in several possible ways, including: 1) increasing the number of antigen reactive cells, 2) decreasing the dose of antigen required for optimal responsiveness, 3) altering the kinetics of the response, and 4) increasing the level of function on the individual cellular level. In vitro assay systems are necessary for these basic studies.

Over the past several years, technological advances have been made which now allow the long-term growth and maintenance of functional antigen-specific T lymphocyte clones in culture. The use of these T cell clones offers numerous advantages over other

in vitro techniques for the study of specific immune responses. One can obtain large quantities of cells responsive not only to a single antigen, but to a single antigenic determinant. Thus, the response to several distinct antigenic determinants of a single infectious agent, or to a determinant common to a class of infectious agents (such as the flaviviruses), can be studied.

Each clone represents a single functional subpopulation. Such homogeneous cell populations allow the dissection of effects of biochemical and cellular influences on individual cell types, and simplify the analyses of the cells themselves. Using T cell clones, the role of other cells of the immune system, such as antigen presenting cells and antibody producing cells can be analyzed with great precision.

In both murine and human systems, T cell clones have been generated against a wide variety of antigens, including cellular alloantigens, tumor antigens, natural and synthetic soluble proteins, and bacterial and viral antigens (1-19). These clones have been used for analyses of fine antigen specificity, histocompatibility antigen (immune response genes) restriction of cellular interactions, cell surface markers, T cell receptor structure, factor production, and mechanisms of regulatory and effector functions. Such analyses have resulted in extremely rapid advancement in understanding the T cell at both cellular and molecular levels.

Studies of modulation of immune function have generally employed unfractionated lymphocyte, T cell, B cell, or macrophage populations. The use of virus-specific T cell clones, however, permits the dissection of effects of biochemical and cellular influences on individual cellular functions. Understanding the role the of each component of the immune response will greatly facilitate the analysis of effects of a single compound or combination of compounds on complex systems of interacting cell types.

The system used here allows comparisons of effects of immunomodulating compounds on responses to various viral antigenic determinants. One compound may improve the response to some antigenic determinants and hinder the response to other antigenic determinants. Clonal populations may be grown in sufficient quantity to assay a variety of compounds, individually or in combination, on essentially identical populations of cells. This system allows analysis of responses of human cells, which may in some cases differ considerably from those of the animal model systems commonly used.

The studies described below employ human T cell clones derived from a single donor and generated against influenza virus strain A/Bangkok. The clones were all generated prior to the initiation of this contract. To facilitate understanding of the

system, however, details of the generation and characterization of these clones are included in this report.

Materials and Methods

Viruses. Formalin fixed preparations of influenza virus strains A/Bangkok RX 73 (H3N2), A/Brazil/X 71 (H1N1), and B/Singapore were a generous gift of Dr. Lance Gordon, Connaught Laboratories, Swiftwater, PA. Before use, virus preparations were dialyzed extensively against PBS to remove formalin. Following dialysis, each preparation was titered in a proliferation assay as described below.

Generation of T lymphocyte clones. Influenza virus-specific T cell clones were generated using slight modifications of our previously described procedure (12). Peripheral blood leukocytes (PBL) ($5x10^5$/ml) were incubated with an optimal dilution of A/Bangkok influenza in 1.5 ml volumes in round bottom 5 ml culture tubes for 5 days. Blast cells were then isolated on Ficoll-Hypaque gradients and cultured for 7 days at a concentration of $1x10^5$/ml with irradiated autologous PBL (feeder cells - $5x10^5$/ml), virus, and conditioned medium (CM - 20%) to expand the influenza-specific T cell population. Cells were then washed and cultured at $1x10^5$/ml with feeders and virus as described above, in the absence of CM, in 5 ml tubes. Three days later, blast cells were again isolated and cloned at limiting dilution. These cultures were carried out in 60 well Terasaki plates in volumes of 0.02 ml/well; T cells were plated at a concentration resulting in 1 cell/3 wells, with each well also containing 10^4 feeders, virus, and 20% CM. Ten days later, the contents of each well containing growing lymphocytes were transferred individually to 0.2 ml flat-bottom wells containing feeders (10^5/well) and flu; CM (final concentration - 20%) was added the following day. After a week, cultures were expanded to 2 ml wells containing 10^6 feeders and flu; again CM was added the next day. One week later, each clone was assayed for influenza-specific proliferative responsiveness. Aliquots of each clone were also frozen and maintained in liquid nitrogen.

Expansion of antigen-specific T cell clones. To expand clonal populations, cells were fed weekly as follows. Cells were cultured at $1x10^5$/ml in RPMI 1640 with 10% A+ plasma and $5x10^5$ irradiated autologous PBL/ml and an optimal concentration of antigen. The following day, CM was added (final concentration 20%). On day 4, half the culture medium was removed and replaced with fresh medium containing 20% CM.

Proliferation assays. T cell clones ($5x10^3$ cells/well) were cultured with irradiated autologous PBL ($25x10^3$ cells/well) in 96 well U-bottom plates in a final volume of 0.2 ml. Each well also contained an appropriate concentration of antigen. Cultures were

incubated for 48 hours, pulsed for 16 hours with 1.0 uCi of ^{3}H-Thymidine and harvested onto glass-fiber filters. Filters were dried and counted using a liquid scintillation counter. All assays were done in triplicate. Cells were cultured in the absence of antigen to provide negative control values, with CM, which nonspecifically stimulates T cell proliferation, as a positive control, and an irrelevant antigen (generally an influenza B strain) as a specificity control.

Conditioned medium production. PBL were cultured at $1x10^{6}$/ml in RPMI containing 1% A+ plasma and 0.1% PHA-P. After 48 hours, supernatants were harvested and filtered. Lots of CM were assayed for their ability to support proliferation of an IL-2 dependent cell line. Acceptable lots were pooled and the volume adjusted for use at a final concentration of 20% in culture.

Analysis of cell surface antigens. Cells were washed and resuspended to 10^{7} cells/ml in RPMI with 10% horse serum and 0.02% Na azide. Aliquots of cells (0.1 ml) were dispensed into 5 ml tubes to which monoclonal antibody was added; the amount of antibody used was determined by titration of each preparation. After 1 hour at 37°C, cells were washed and incubated with goat anti-mouse Ig for 30 min. at 37°C. Cells were again washed and then incubated with fluorescein-conjugated rabbit anti-goat IgG for 30 min. at 37°C. After two washes, cells were fixed in 2% paraformaldehyde and analyzed for fluorescence using a fluorescence activated cell sorter.

Virus-Specific Cytotoxicity. Target cells. Target cells are EBV transformed cells derived from the same donor as was used to generate the T cell clones to be assayed. The use of EBV transformed cells as targets allows the detection of CTL which are HLA Class II restricted; cytotoxic effects of these Class II restricted clones often cannot be detected using PHA blasts as targets, due to insufficient levels of expression of Class II antigens. At t=-24 hrs., target cells were diluted to 1×10^{5} cells/ml in RPMI 1640, 10% FCS, $\pm$ influenza A (final dilution - 1:250) and incubated at 37°, 5% CO_2. For use as a specificity control, target cells are incubated with medium rather than virus. At t= 0, target cells were harvested and viable cells purified on a Ficoll-Hypaque gradient. Cells at the interface were collected and washed 3 times in RPMI 10% FCS. Cells were pelletted (up to 10^{7} cells/tube) and 200 uCi ^{51}Cr (in 0.1 ml) was added. Cells were resuspended and incubated at 37°, 5% CO_2, for 1 hr., mixing every 15 min. Cells were washed 3 times in RPMI 10% FCS, counted, and resuspended at 1×10^{5} cells/ml.

Effector cells. T cell clones were used as killers 7 days after feeding with antigen and antigen presenting cells. Cells were washed once with RPMI 10% FCS, counted, and resuspended to required concentration (determined for each clone to yield

effective lysis of 10^4 targets; for most clones, 10^5 killer cells/well was optimal)

Assay. Assays were carried out in 96 well U bottom plates. 0.1 ml of killer cells and 0.1 ml of target cells were dispensed into each well. As controls, targets were incubated with 0.1 ml of medium (spontaneous ^{51}Cr release), or with 0.1 ml 0.1 N HCl (maximum ^{51}Cr release). Plates were centrifuged at 1000 rpm for 30 sec. to lightly pellet the cells. Plates were then incubated at 37°, 5% CO_2, for 4 hrs, centrifuged at 1000 rpm for 2 min., harvested using Skatron supernatant harvesters, and counted. % specific lysis was calculated as:

$$\frac{\text{Experimental cpm - Spontaneous cpm}}{\text{Maximum cpm - Spontaneous cpm}} \times 100$$

For assays in which immunomodulators were added to killer or target cells on the day of the assay, cells were added at 2X the normal concentration in half the normal volume to allow addition of the modulators without alteration of the total assay volume.

Results

Characterization of T cell clones

Screening of donors. To identify an appropriate donor for generation of T cell clones, PBL from several healthy volunteers were assayed for in vitro proliferative responses to influenza virus. PBL ($5x10^5$/well) were cultured with medium or dilutions of influenza A/Bangkok ranging from 0.1 to 50 HAU/ml for 6 days, after which incorporation of ^3H-thymidine was measured. The results of one such experiment are shown in Table 1; for simplicity, only one virus concentration is presented. Donor 945 clearly gave higher proliferative responses in this and other experiments and was therefore used for the generation of clones for the studies described here.

Determination of optimal virus dose for in vitro studies. Each preparation of virus must be tested for optimal dilution for use in generating and maintaining clones and for proliferation assays. PBL from donor 945 were cultured with varying dilutions of virus for 6 days and assayed for proliferation as described above. Data from the most recent titration of influenza A/Bangkok are presented in Table 2; for this preparation, a 1:1000 dilution results in optimal proliferation. Similar results (not shown) were obtained with strains A/Brazil and B/Singapore.

Antigen specificity of T lymphocyte clones. A large number of T lymphocyte clones were generated as described above, using

PBL obtained from donor 945. Of these, 6 were chosen for the initial phases of these studies. To determine their antigen specificities, clones were incubated with irradiated autologous PBL, which serve as antigen presenting cells, and medium (background), 20% conditioned medium (a nonspecific stimulus which serves as a positive control), or optimal concentrations of virus in a 2 day proliferation assay. As can be seen in Table 3, all 6 clones proliferated well to conditioned medium and therefore were healthy and able to divide after nonspecific stimulation. Clones TFE96 and TFE97 were able to respond to an H3N2 strain (A/Bangkok) of flu, but not to the H1N1 strain A/Brazil, indicating that these clones recognize determinants on either the hemaglutinin or neuraminadase components of the virus. Clones TFE40, TFE89, and TFE102 all responded to both strains A/Bangkok and A/Brazil, indicating that these clones are specific for antigenic determinants shared by A strain influenza viruses. Clone TFE88 responded to neither A/Bangkok nor A/Brazil; it was included in some experiments as a control for nonantigen specific effects of immunomodulators. In addition, but not shown here, all clones were assayed for responsiveness to strain B/Singapore and in all cases incorporated less than 100 cpm.

Kinetics of responses of T cell clones. To determine the day of peak proliferative response of the T cell clones used here, clones were cultured with feeders and virus and pulsed with ^{3}H-thymidine after 2, 3, or 4 days of culture. The data in Table 4 demonstrate that clones TFE40, TFE96, and TFE102 responded optimally 2 days after initiation of cultures. Clone TFE97 responded optimally on day 3, but displayed only slightly lower responsiveness on day 2. All assays described below were therefore pulsed on day 2 unless otherwise indicated.

Cell surface phenotype of T cell clones. T cell clones were stained with monoclonal antibodies against the CD4 (OKT 4) and CD8 (OKT 8) determinants followed by fluorescein conjugated goat anti-mouse Ig antibodies and analyzed using a fluorescence activated cell sorter. The results in Table 5 indicate that all of these clones express the CD4 antigen and are negative for the CD8 marker.

Virus-specific cell mediated cytotoxicity. Of the large number of virus-specific T cell clones originally generated, four cytotoxic clones were used in these studies (Table 6). In all assays, target cells which had been incubated with medium rather than Influenza virus were included as a specificity control. Any killing due to recognition of EBV antigens or other cell surface antigens would be seen as killing of targets incubated with medium as well as targets incubated with virus; using T cell clones as cytotoxic effectors, this nonspecific lysis has not been seen. In assays of immunomodulators, targets incubated with medium also served to control for any nonspecific effects of these compounds on either the effector or target cells.

Evaluation of immunomodulators

A total of 19 compounds (18 potential immunomodulators and 1 placebo) were supplied for testing under this contract. These compounds and their AVS numbers are listed in Table 7.

The first compound to be tested was CL 246,738. T cell clones were cultured with a series of ten-fold dilutions of the modulator. The modulator was included from the beginning of the culture and proliferation was assayed on day 2. The results of the first assay (Table 8) demonstrated that this compound was toxic to the cells at a concentration of 10 ug/ml, with incorporation of ^{3}H-TdR into DNA eliminated, and lysis of cells observed on visual inspection of cultures. Partial inhibition of cell division in response to CM and to flu compared to cultures containing no modulator was observed when the compound was included at a final concentration of 1 ug/ml. At lower concentrations of modulator, however, an increase in the response to flu A was seen. Proliferation in response to the irrelevant antigen, flu B, was unaffected, and nonspecific responsiveness to CM was only marginally increased. The response of the nonspecific clone, TFE 88 was not appreciably altered under these culture conditions. It thus appears that only the response to specific antigen is enhanced. In further experiments similar results were seen; results with antigen and modulator concentrations giving optimal enhancement of proliferation are shown in Table 9. It should be noted that the antigen concentration which results in optimal stimulation appears to be different for different clones.

The effect of CL 246,738 on virus-specific cytotoxicity was then tested over a wide range of concentrations. Initially, effectors were incubated with CL 246,738 for 1 hour before target cells were added; partial inhibition of killing was seen at 10^{-1} ug/ml and killing was completely eliminated at 1 ug/ml (Table 10). Proliferation and cytotoxicity assays were carried out side-by-side, as described above. The data shown in Table 11 demonstrate that at 10^{-1} ug/ml, the cytotoxic response was less than half of the control level for each clone, while the proliferative response was enhanced relative to the control response, as was seen in initial experiments with this compound. The kinetics of inhibition of CTL responses by CL 246,738 was also examined. Killing by TFE 89 (Table 12) was slightly inhibited when 10^{-1} ug/ml of CL 246,738 was added at the same time as target cells, while a 1 hour or 24 hour preincubation resulted in a much greater inhibition. The effect of 1 ug/ml was similar with 0 or 1 hour incubation, while a 24 hour incubation resulted in total elimination of killing. In contrast, killing by clone TFE 23 (Table 13) was inhibited to a greater extent by 10^{-1} ug/ml at 1 hour than at 24 hours, while 1 ug/ml totally eliminated killing with either incubation time.

The effect of OK-432 on virus-specific proliferation was also tested over a concentration range from 10 ug/ml to 10^{-6} ug/ml with virus dilutions ranging from 1:1000 to 1:16,000. Optimal enhancement was seen in all cases with a virus dilution of 1:2000; the concentration of OK-432 which induced optimal enhancement, however, varied for each clone (Table 14). To examine the effects of this compound on virus-specific cytotoxicity, OK 432 was incubated with cytotoxic clones for 1 hour before target cells were added (Table 15). Killing by TFE 18 was enhanced over the entire range of concentrations tested. The level of killing by TFE 23 was also enhanced at most concentrations of OK 432, but to a slightly lesser extent. The responses of TFE 79 and TFE 89 were variable, with only modest enhancement and modest inhibition observed.

Dramatic differences among the clones were seen in the effects of recombinant human gamma interferon (AVS 2250) on antigen-specific proliferation (Table 16). Clone TF E89 displayed enhanced proliferation at low interferon concentrations and decreased proliferation at relatively high interferon concentrations. Optimal enhancement of proliferation of clone TF E96 was seen at intermediate concentrations, while proliferation of clones TF E97 and TF E102 was optimally enhanced at high concentrations of interferon. Decreased proliferation was not seen at any interferon concentration with any clone other than TF E89. These effects were seen at all antigen dilutions tested, although they were most dramatic at suboptimal antigen concentrations. Both the inhibition and enhancement were seen only with antigen-specific responses; no effect was seen on nonspecific proliferation induced by conditioned medium or on negative control responses. That inhibition of proliferation was not due to toxicity or interference with thymidine uptake by the cells was confirmed by the results with conditioned medium as well as by trypan blue viability counts of the cells after culture.

To study the effects of interferon on expression of surface markers, clones were incubated with feeder cells and antigen for two days in the presence or absence of 1 ug/ml of interferon prior to cell sorter analysis (Tables 17 and 18). This timing and concentration of interferon were chosen since these conditions are those under which we found an effect of interferon on antigen-specific proliferation. After incubation with antigen and interferon, clone TF E89, which previously displayed decreased antigen-specific proliferation in response to 1 ug/ml of interferon, was found to express slightly decreased levels of CD4 antigens relative to cells incubated with antigen in the absence of interferon. Clone TF E97, which displayed enhanced antigen-specific proliferation in the presence of 1 ug/ml interferon, was found to have increased expression of CD4 and IL-2 receptor after incubation with antigen and interferon relative to cells incubated with antigen alone. Clone TF E102 also showed

enhanced CD4 expression after incubation with interferon; expression of IL-2 receptor, however, was not enhanced in this clone. Each clone thus displays a unique pattern of alteration of surface marker expression in response to gamma interferon.

A series of experiments was carried out to examine the effects of recombinant leukocyte A interferon (AVS 4625) on proliferation of influenza virus-specific T cell clones. Initially, AVS 4625 was tested over a range from 10^{-2} to 10^{4} U/ml. The results of these assays are shown in Tables 19a-19e. Some enhancement of antigen-specific proliferation was seen with each clone under particular conditions (which differed for each clone); only with TFE 96, however, could enhancement be detected over a range of concentrations of AVS 4625 and antigen (Table 1c). At concentrations of 10^{3} and 10^{4} U/ml, AVS 4625 consistently suppressed proliferation of all 5 clones; this supression was seen when proliferation was induced by specific antigen (Flu A) as well as when proliferation was nonspecifically induced by conditioned medium (CM). Similar levels of suppression were seen with optimal antigen concentrations and with CM (15%-35% of control response remaining), with greater suppression of responsiveness at lower antigen concentrations (up to 98% inhibition). To further examine enhancement of proliferation by AVS 4625, this compound was included in another set of experiments at doses from 10^{-4} to 10^{2} U/ml (Tables 20a-20e). In these experiments, enhancement of antigen-specific (but not CM-induced) proliferation could be detected over the entire range of antigen concentrations tested. This enhancement was seen primarily, but not exclusively, at lower concentrations of AVS 4625, and again, maximal enhancement (up to 4.3 fold) was seen at lower antigen doses. The effects of AVS 4625 on clonal cytotoxicity are shown in Table 21. At doses of 10^{-1} U/ml or less, cytotoxicity was either slightly inhibited or was unchanged. Doses of 1 U/ml or more, however, caused slight enhancement of the response of clone TFE 23, which displayed a high control level of killing, and much greater enhancement of the levels of killing by clones TFE 79 and TFE 89, which displayed lower control cytotoxicity.

The effects of recombinant hybrid human interferon alpha BD (AVS 5311) on proliferation of virus-specific T cell clones are shown in Tables 22a-22e. As was seen with AVS 4625 (recombinant leukocyte A interferon), both antigen-specific proliferation and nonspecific proliferation induced by conditioned medium were suppressed by high concentrations (10^{4} U/ml) of interferon. At lower concentrations of interferon, modest enhancement was seen only in isolated cases; our experience with AVS 4625 indicates that concentrations even lower than the lowest tested here (10^{-2} U/ml) may be necessary to see consistent enhancement of proliferation. When clone TFE 89 was incubated with AVS 5311 for 1 hour prior to addition of Flu A coated targets, enhancement of killing is seen at concentrations of 10 U/ml or more (Table 23).

Addition of AVS 5311 at the same time as target cells results in a slightly lower degree of enhancement, but optimal enhancement is seen lower concentrations of interferon (Table 24).

Poly ICLC (AVS 1761) was tested for effects on T cell clonal proliferation over concentrations ranging from 10 to 10^{-5}ug/ml (Tables 25a-25e). As with AVS 4625, modest enhancement of antigen-specific proliferation was seen under selective conditions with each clone; the response of TFE 96 to virus at 1:16000, however, was enhanced more than 3 fold (Table 25c). High concentrations of Poly ICLC (1 and 10 ug/ml) suppressed the antigen-specific proliferative responses of all of the clones tested. Here again, the responses to lower antigen concentrations were suppressed to a much greater extent than was proliferation to optimal antigen concentration. The proliferative responses to suboptimal antigen doses were also inhibited by lower concentrations of Poly ICLC. As contrasted with AVS 4625, however, Poly ICLC only marginally inhibited the nonspecific proliferative response to conditioned medium. The effect of Poly ICLC on virus-specific cytotoxicity was similar to that seen with AVS 4625; enhancement of specific killing by clone TF E89 was seen even at concentrations of Poly ICLC which markedly inhibited virus-specific proliferation by this clone (Table 26).

Poly I.Poly C 12U (AVS 2149) was tested for effects on T cell clonal proliferative responses over the same range of concentrations and of antigen dilutions as were used for Poly ICLC; results are presented in Tables 27a-27d. Enhancement of proliferation of all clones was seen with AVS 2149 at 1 ug/ml at antigen dilutions ranging from 1:4000 to 1:16,000. Some enhancement was also seen at lower concentrations of AVS 2149 and/or higher concentrations of virus, but this was not consistent among the clones.

AVS 2149 was also tested for effects on virus-specific cytotoxicity. When cytotoxic effectors and drug were mixed at the time of target cell addition, some decrease in killing was seen (Table 28). When effectors and drug were incubated together for 1 hour prior to addition of target cells (Table 29), killing was inhibited to a much greater extent. In both situations, greater inhibition was seen at higher drug concentrations. Greater inhibition was also seen in clones which displayed higher control levels of killing.

Effects of liposomal muramyl tripeptide (AVS 2933) and control placebo liposomes (AVS 4726) on T cell clonal proliferation are shown in Tables 30a,b-34a,b. In each case the effects of MTP and the placebo were tested simultaneously with each clone. Modest effects of both MTP and the placebo on the nonspecific response to conditioned medium were seen; in some cases proliferation was slightly enhanced, in others, slightly

inhibited. At the highest concentrations tested, both MTP and placebo liposomes caused marked inhibition of antigen-specific proliferative responses at all antigen concentrations. The degree of inhibition seen with MTP, however, was much greater than that seen with the placebo; this is particularly evident at lower antigen concentrations. No significant effect of MTP or placebo liposomes on virus-s[ecific cytotoxicity was detected at any concentration tested (Table 35).

Two related compounds, 1-isobutyl-4(1H)-imidazo[4,5-C] quinolinamine (AVS 1018) and its HCl salt (AVS 1300) were assayed in parallel. No significant effect of AVS 1018 on antigen-specific proliferation was seen with clones TF E40 or TF E102 at any antigen concentration or drug concentration, and only modest enhancement was detected with TF E89. Proliferation of clones TF E96 and TF E97 was enhanced up to 2 fold by drug concentrations in the range of 10^{-3} to 10^{-5} ug/ml at suboptimal antigen concentrations (Table 36). A different pattern was seen with AVS-001300 (Table 37). This compound induced enhanced proliferation of all clones tested. Optimal enhancement was seen at low antigen concentrations and with drug concentrations in the range of 10^{-1} to 10^{-3} ug/ml, but some degree of enhancement was seen over a relatively wide range of drug concentrations.

Inhibition of killing was seen when either AVS 1018 (Table 38) or AVS 1300 (Table 39) were incubated with effectors for 1 hour prior to addition of target cells. AVS 1018 inhibited killing by clones TFE 18 and TFE 23, which displayed high control levels of killing, and by TFE 89, which in this experiment killed at an intermediate level; the drug dose which maximally inhibited was different for each clone. The low level of killing by TFE 79 appeared to be enhanced by AVS 1018 at 1 ug/ml. A different pattern was seen with AVS 1300. Killing by clones TFE 18 and TFE 79 was inhibited by AVS 1300 at all concentrations tested. Virus-specific killing by clones TFE 23 and TFE 89, however, was moderately inhibited by some concentrations of AVS 1300 and modestly enhanced by others.

In proliferation assays, AVS 2880 induced significantly enhanced proliferation in only some of the clones; the most dramatic enhancement was seen with clone TF E97 at suboptimal antigen concentrations (Table 40). This compound alone caused significant release of ^{51}Cr from target cells, therefore it was not possible to assay its effects on virus-specific cytotoxicity.

Six related compounds, ABPP (AVS 2776), AIPP (AVS 2777), ABMP (AVS 2778), ACPP (AVS 3587), ABMFPP (AVS 3588), and ACDFPP (AVS 3589) all required high concentrations of DMSO to dissolve, but remained in solution when diluted in tissue culture medium for assay. All cultures therefore contained DMSO at the same final concentration (generally 1%) to control for effects of the solvent on the clones. AVS 2776 induced enhanced proliferation

in four of the five clones tested (Table 41). This enhancement was seen only at low antigen doses; the modulator dose required for optimal enhancement, however, was variable. Enhancement generally was in the range of 1.7 to 3 fold, but in the case of clone TFE102 at a 1:16,000 dilution of virus, up to 14.8 fold increase in response was seen. AVS 2777 was able to increase proliferation of all clones tested (Table 42). Optimal conditions varied, both in terms of antigen dilution and modulator concentration, and in all cases enhancement was in the range of 1.5 to 4 fold. Similar results were obtained with AVS 2778 (Table 43) and AVS 3587 (Table 44); neither of these, however, induced enhanced proliferation in all five clones. No effects on proliferation of any of the clones were seen with AVS 3588 or AVS 3589 under any conditions tested. The relatively high DMSO concentrations required to keep these compounds in solution resulted in considerable leakage of ^{51}Cr from target cells, making cytotoxicity assays with these agents impossible.

Conclusions

A series of human T cell clones generated against Influenza A virus (strain A/Bangkok RX 73) was used as a model system to evaluate immunomodulatory potential of a variety of compounds. Included in the group were clones specific for various viral proteins as well as representatives of different functional T cell subpopulations.

Many of the compounds tested affected antigen-specific proliferation of some or all of the T cell clones, and both enhancement and suppression of proliferation were seen. Some compounds affected proliferation under the same conditions for all clones, while with others, optimal effects were seen with different concentrations of virus and/or immunomodulator for each clone. The effects seen were generally most profound on suboptimal control responses. Of particular interest was gamma interferon, which under identical conditions enhanced the proliferation of some clones while inhibiting proliferation of others. In most cases, only virus-specific proliferation was affected, but a few of the compounds altered nonspecific proliferation induced by IL-2 containing conditioned medium.

In assays of virus-specific cell mediated cytotoxicity, most compounds which enhanced proliferative responses suppressed cytotoxicity under similar conditions, while most of those which suppressed proliferation enhanced cytotoxicity. Poly ICLC (only at relatively low concentrations) and OK-432, however, were found to enhance both virus-specific proliferation and cytotoxicity under the same conditions. As was seen in proliferation assays, there was variation among the clones in their response to different compounds.

The unique patterns of responsiveness of different T cell clones to many of the immunomodulatory compounds tested indicate that such a system can be useful in dissecting the basic effects of such agents on individual components of the immune response. Since differences were seen both with different T cell functional subpopulations as well as among clones with specificity for various viral antigens, it will be critical in future studies to use clones generated against viruses of military interest. It should also be noted that all of the clones used here were generated from a single donor, thus no assessment of individual variation in responsiveness to either the virus or the immunomodulators was possible. The ultimate value of studies of potential immunomodulators will not be in the data generated alone but in such data in conjunction with other studies such as those which employ in vivo animal models.

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APPENDIX I

TABLES 1 - 44

Donor	Medium	Flu A/Bangkok
15	1,362 (247)	4,130 (814)
20	686 (175)	6,284 (2035)
44	570 (113)	16,625 (1132)
127	635 (104)	3,449 (1523)
769	305 (39)	15,123 (1017)
945	404 (27)	19,984 (135)
1719	401 (43)	8,175 (123)
2156	295 (44)	6,313 (925)

Table 1. Influenza Virus-Specific Proliferative Response of PBL from 8 Normal Donors.

PBL from healthy volunteers were cultured for 6 days with influenza virus (5 HAU/ml). Cultures were pulsed with ^{3}H-thymidine for the final 18 hours of culture and incorporation of radiolabel into DNA measured. Data are presented as mean cpm (SEM); all cultures were done in triplicate.

Flu dilution	cpm (S.E.)
medium	2557 (559)
1:250	4884 (453)
1:500	7302 (1645)
1:1000	10,548 (1416)
1:2000	4457 (1436)
1:4000	4329 (620)
1:8000	5645 (952)
1:16,000	2140 (774)

Table 2. Dose-Response of PBL to Influenza A/Bangkok.

PBL ($5x10^5$/well) from donor 945 were cultured with the indicated dilutions of virus for 6 days and pulsed with ^{3}H-thymidine for the final 18 hours of culture. Data are the mean of triplicate cultures (SEM).

Clone	Medium	CM	Flu A/Bangkok (H3N2)	Flu A/Brazil (H1N1)
TFE40	161 (50)	18,578 (642)	21,337 (240)	26,025 (467)
TFE88	69 (24)	9,394 (755)	103 (45)	157 (36)
TFE89	52 (19)	7,573 (347)	10,906 (52)	12,517 (392)
TFE96	37 (3)	7,342 (578)	6,153 (606)	79 (29)
TFE97	31 (5)	6,415 (145)	6,919 (551)	502 (73)
TFE102	41 (16)	6,589 (411)	10,031 (586)	15,783 (571)

Table 3. Antigen Specificity of T Lymphocyte Clones.

T cell clones ($1x10^4$/well) were cultured with autologous irradiated feeders ($2.5x10^4$/well) and medium, 20% CM, or optimal dilutions of virus as indicated. After 2 days cultures were pulsed with ^{3}H-thymidine for 18 hours and incorporation of label into DNA was measured. Data are presented as mean of triplicate cultures (SEM).

Clone	Day 2	Day 3	Day 4
TFE40	4295 (725)	3037 (423)	NT
TFE96	2121 (345)	1469 (215)	470 (33)
TFE97	4196 (324)	5293 (492)	3631 (585)
TFE102	1421 (154)	1273 (360)	659 (131)

Table 4. Kinetics of Proliferative Response of T Cell Clones.

T cell clones ($1x10^4$/well) were cultured with irradiated autologous feeders ($2.5x10^4$/well) and influenza A/Bangkok (1:1000). After the indicated times cultures were pulsed with ^{3}H-thymidine for 18 hours and incorporation of label into DNA was measured. Data is presented as mean cpm for triplicate cultures (SEM). Background for clones cultured with feeders in the absence of virus in all cases was less than 100 cpm.

	% Positive	
Clone	CD 4	CD 8
TFE40	99.4	6.3
TFE88	95.1	3.5
TFE89	97.5	2.2
TFE96	95.9	3.7
TFE97	98.9	7.4
TFE102	98.3	1.9

Table 5. Expression of Cell Surface Markers on T Cell Clones.

Cells were incubated with OKT4 (CD4) or OKT8 (CD8) monoclonal antibodies followed by fluorescein-conjugated goat anti-mouse Ig antibodies. Stained cells were analyzed using a fluorescence activated cell sorter.

Clone	Medium	Flu A 1:250
TFE 18	1.0	52.9
TFE 23	0.7	46.4
TFE 79	0	19.8
TFE 89	0.8	19.1

Table 6. Virus-Specific Cytotoxicity Mediated by T Cell Clones

Clones were assayed for the ability to kill syngeneic EBV transformed target cells which had been incubated for 24 hours with either medium or virus as indicated. Results are expressed as % specific lysis.

AVS Number	Compound
--	OK-432
1968	CL 246,738
2269	Tumor Necrosis Factor
2350	Human Recombinant Interferon Gamma
4625	Recombinant Leukocyte A Interferon
5311	Recombinant Hybrid Human Interferon Alpha BD
1018	1-isobutyl-4(1H)-imidazo[4,5-C]quinolinamine
1300	1-isobutyl-4(1H)-imidazo[4,5-C]quinolinamine, HCl salt
2776	2-amino-5-bromo-6-phenyl-4(3H)-pyrimidinone (Bropirimine, ABPP)
2777	2-amino-5-iodo-6-phenyl-4(3H)-pyrimidinone (AIPP)
2778	2-amino-5-bromo-6-methyl-4(3H)-pyrimidinone (ABMP)
2880	DL-2,3,5,6,7,8-hexahydro-8,8-dimethoxy-2-phenylimidazo[1,2-A]pyridine hydrochoride
3587	ACPP
3588	ABMFPP
3589	ACDFPP
2149	PolyI.PolyC12U
1761	PolyICLC
2933	Liposomal Muramyl Tripeptide (Muramyl Tripeptide Phosphatidylethanolamine)
4726	Liposome (Placebo for AVS 2933)

Table 7. Compounds provided for testing of immunomodulatory activity.

Modulator Concentration	Medium	CM	Flu A/Bangkok 1:500	1:1000	1:2000
0	32 (9)	6810 (463)	3862 (348)	2419 (241)	1502 (514)
10^{-3} ug/ml	23 (10)	7915 (521)	4480 (202)	2441 (389)	1765 (101)
10^{-2} ug/ml	32 (2)	7427 (314)	4561 (451)	2693 (121)	1936 (240)
10^{-1} ug/ml	49 (26)	6922 (237)	5054 (291)	3425 (298)	2678 (596)
1 ug/ml	19 (8)	2474 (170)	1481 (163)	1008 (112)	539 (133)
10 ug/ml	15 (4)	16 (5)	11 (1)	25 (6)	19 (1)

Table 8. Toxicity of Modulator CL 246,738.

Clone TFE97 ($1x10^4$ cells/well) was cultured with irradiated autologous feeders ($2.5x10^4$/well) and the indicated antigen, along with the indicated concentration of CL 246,738. After 48 hours, cultures were pulsed for 18 hours with ^{3}H-thymidine and incorporation of radiolabel into DNA was measured. Results are expressed as mean cpm for triplicate cultures (SEM).

Clone	CL 246,738 Concentration	Medium	Flu A 1:2000	Flu A 1:16,000
TFE40	0	43 (14)	3232 (186)	757 (134)
	10^{-1} ug/ml	25 (8)	3477 (283)	1645 (228)
TFE89	0	55 (19)	4186 (133)	948 (41)
	10^{-1} ug/ml	54 (22)	4209 (369)	1642 (158)
TFE97	0	28 (6)	3128 (313)	611 (157)
	10^{-1} ug/ml	37 (10)	4143 440	824 (180)

Table 9. Effect of CL 246,738 on Proliferation of Virus-Specific T Cell Clones.

T cell clones ($1x10^4$/well) were cultured with irradiated autologous feeders ($2.5x10^4$/well) and the indicated concentrations of antigen and CL 246,738. After 48 hours, cultures were pulsed with ^{3}H-thymidine for 18 hours and incorporation of label into DNA was measured. Results are expressed as mean cpm of triplicate cultures (SEM).

[CL 246,738]	TFE 18	TFE 23	TFE 79	TFE 89
0	36.0	49.1	3.4	10.1
10^{-7} ug/ml	43.1	49.6	3.8	10.2
10^{-6}	41.4	48.4	2.2	9.0
10^{-5}	40.9	53.5	1.5	9.1
10^{-4}	38.2	55.5	2.7	8.1
10^{-3}	47.3	53.8	6.2	10.7
10^{-2}	37.6	49.4	4.3	9.5
10^{-1}	23.7	31.1	2.4	4.1
1	2.3	2.5	0.2	1.8

Table 10. Effect of CL 246,738 on Virus-Specific Cytotoxicity

Effector cells were incubated with the indicated concentrations of CL 246,738 for 1 hour before addition of target cells. Results are expressed as % specific lysis.

Clone	[CL 246,738]	Prolif.*	% Specific Lysis
TFE 23	0	13,697	58.6
	10^{-6}	14,876	57.2
	10^{-5}	14,256	49.3
	10^{-4}	14,124	40.1
	10^{-3}	15,459	54.5
	10^{-2}	16,404	48.6
	10^{-1}	15,495	20.7
	1	96	6.2
	10	33	7.4
TFE 89	0	11,306	26.6
	10^{-4}	11,695	22.2
	10^{-3}	12,865	22.1
	10^{-2}	13,181	16.0
	10^{-1}	14,308	12.2
	1	101	8.5
	10	59	11.1

Table 11. Effect of CL 246,738 on Proliferation and Cytotoxicity

* Flu A 1:4000

[CL 246,738]	0 hours	1 hour	24 hours
0	15.1	16.5	10.9
10^{-6} ug/ml	17.7	17.2	13.5
10^{-5}	15.7	17.1	11.8
10^{-4}	17.0	17.0	11.6
10^{-3}	15.7	19.5	12.4
10^{-2}	18.0	17.4	10.4
10^{-1}	11.1	7.6	4.9
1	7.7	7.5	-0.3

Table 12. Kinetics of CL 246,738 Effect on Virus-Specific Cytotoxicity

Clone TFE 89 was preincubated with the indicated concentrations of CL 246,738 for the indicated length of time prior to the addition of influenza A coated target cells. Results are expressed as % specific lysis.

[CL 246,738]	1 hour	24 hours
0	41.0	38.1
10^{-6} ug/ml	46.3	47.3
10^{-5}	43.4	46.3
10^{-4}	42.1	44.0
10^{-3}	47.3	44.1
10^{-2}	40.1	47.8
10^{-1}	10.7	24.5
1	1.2	-0.8

Table 13. Kinetics of CL 246,738 Effect on Virus-Specific Cytotoxicity

Clone TFE 23 was preincubated with the indicated concentrations of CL 246,738 for the indicated lengths of time prior to the addition of influenza A coated target cells. Results are expressed as % specific lysis.

Clone	Modulator	Medium	Flu A 1:2000
TFE89	-	72 (7)	3325 (101)
	10^{-3} ug/ml	68 (10)	4805 (293)
TFE96	-	83 (17)	3277 (107)
	10^{-1} ug/ml	67 (8)	4099 (98)
TFE97	-	95 (27)	2120 (194)
	10^{-6} ug/ml	117 (2)	2799 (425)
TFE102	-	63 (5)	2531 (212)
	10^{-3} ug/ml	47 (10)	3651 (365)

Table 14. Effect of Modulator OK432 on Proliferation of Virus-Specific T Cell Clones.

T cell clones ($1x10^4$ cells/well) were cultured with irradiated autologous feeders ($2.5x10^4$/well) and the indicated concentrations of antigen and OK432. After 48 hours, cultures were pulsed with ^3H-thymidine for 18 hours and incorporation of label into DNA was measured. Results are expressed as mean cpm for triplicate cultures (SEM).

[OK 432]	TFE 18	TFE 23	TFE 79	TFE 89
0	58.1	51.1	9.5	22.0
10^{-6} ug/ml	73.6	57.2	6.7	17.7
10^{-5}	70.3	50.7	7.5	18.2
10^{-4}	69.2	54.7	12.0	22.2
10^{-3}	68.6	49.4	5.9	24.0
10^{-2}	62.9	6 2	8.7	22.3
10^{-1}	65.8	57.3	6.7	21.8
1	64.6	55.8	11.4	19.7
10	74.5	56.6	9.2	23.0

Table 15. Effect of OK 432 on Virus-Specific Cytotoxicity

T cell clones were incubated with the indicated concentrations of OK 432 for 1 hour prior to the addition of target cells. Results are expressed as % specific lysis.

IFN Conc.	E40	E89	E96	E97	E102
0	286 (108)	1875 (149)	1361 (360)	310 (29)	110 (14)
10^{-6} ug/ml	206 (108)	2833 (432)	1838 (203)	449 (67)	323 (48)
10^{-5} ug/ml	177 (79)	2525 (158)	2507 (261)	667 (100)	175 (47)
10^{-4} ug/ml	447 (245)	2235 (211)	3575 (860)	714 (118)	317 (57)
10^{-3} ug/ml	303 (83)	2275 (143)	1807 (108)	614 (70)	161 (79)
10^{-2} ug/ml	208 (79)	1892 (209)	1785 (274)	767 (34)	239 (61)
10^{-1} ug/ml	396 (61)	1253 (143)	1968 (377)	1152 (171)	180 (48)
1 ug/ml	515 (169)	800 (102)	1877 (108)	879 (162)	347 (77)

Table 16. Effect of Gamma Interferon on Proliferation of Virus-Specific T Cell Clones.

T cell clones ($1x10^4$ cells/well) were cultured with irradiated autologous feeders ($2.5x10^4$/well), a suboptimal concentration of influenza virus (1:8000), and the indicated concentration of gamma interferon. After 48 hours, cultures were pulsed with ^{3}H-thymidine for 18 hours and incorporation of label into DNA was measured. Results are expressed as mean cpm for triplicate cultures (SEM). Clones incubated with medium rather than virus incorporated less than 40 cpm.

Clone	Interferon	% Positive	Mean Fl Channel
E40	-	50.2	244
	+	46.3	278
E89	-	62.5	367
	+	47.8	278
E96	-	53.6	254
	+	42.8	235
E97	-	61.5	295
	+	86.0	619
E102	-	62.8	365
	+	79.8	492

Table 17. Effect of Gamma Interferon on Cell Surface Expression of CD4.

T cell clones ($1x10^5$ cells/ml) were cultured with irradiated autologous feeders ($2.5x10^5$/ml), a suboptimal concentration of influenza virus (1:8000), and 1 ug/ml gamma interferon. After 48 hours, cells were analyzed for expression of surface CD4.

Clone	Interferon	% Positive	Mean Fl Channel
E40	-	15.0	178
	+	14.7	186
E89	-	14.9	104
	+	11.5	173
E96	-	14.9	198
	+	22.0	223
E97	-	5.5	258
	+	40.5	254
E102	-	11.0	192
	+	10.2	204

Table 18. Effect of Gamma Interferon on Cell Surface Expression of IL-2 Receptors.

T cell clones ($1x10^5$ cells/ml) were cultured with irradiated autologous feeders ($2.5x10^5$/ml), a suboptimal concentration of influenza virus (1:8000), and 1 ug/ml gamma interferon. After 48 hours, cells were analyzed for expression of surface IL-2 receptors.

[AVS 4625]	Medium	CM	Flu A 1:1000	1:2000	1:4000	1:8000	1:16000	Flu B 1:1000
0	58	6552	4845	5058	3637	1453	771	45
10^{-2}	24	6241	5667	3309	2904	1895	726	22
10^{-1}	23	7103	5536	3433	3042	1661	993	44
1	31	6023	5696	4851	3457	2257	720	21
10	29	4559	7099	4015	3667	1159	698	30
10^2	33	3532	4753	3965	2022	1211	407	21
10^3	24	2725	2971	1140	1313	605	165	17
10^4	27	1722	892	542	133	84	40	13

Table 19a. Effect of Leukocyte A Interferon (AVS 4625) on Proliferation of Clone TFE 40.

T cell clones were incubated with irradiated autologous feeders, antigen, and the indicated concentrations (U/ml) of AVS 4625. After 48 hours, cultures were pulsed with ^{3}H-thymidine for 18 hours and incorporation of label into DNA was measured. Results are expressed as mean cpm for triplicate cultures.

[AVS 4625]	Medium	CM	Flu A 1:1000	Flu A 1:2000	Flu A 1:4000	Flu A 1:8000	Flu A 1:16000	Flu B 1:1000
0	16	5929	6877	5623	4411	2425	1061	17
10^{-2}	25	5137	7252	5609	3967	1755	852	58
10^{-1}	85	5079	7644	4604	3595	1931	791	21
1	21	4541	6454	5385	3404	2277	1223	45
10	39	4097	6538	5665	4316	1704	901	19
10^2	23	3357	5419	2843	2348	973	360	31
10^3	17	2025	3317	2449	996	267	94	36
10^4	14	1384	1681	724	184	57	35	18

Table 19b. Effect of Leukocyte A Interferon (AVS 4625) on Proliferation of Clone TFE 89.

T cell clones were incubated with irradiated autologous feeders, antigen, and the indicated concentrations (U/ml) of AVS 4625. After 48 hours, cultures were pulsed with ^{3}H-thymidine for 18 hours and incorporation of label into DNA was measured. Results are expressed as mean cpm for triplicate cultures.

[AVS 4625]	Medium	CM	Flu A 1:1000	Flu A 1:2000	Flu A 1:4000	Flu A 1:8000	Flu A 1:16000	Flu B 1:1000
0	39	5520	5431	4755	4306	2410	2142	57
10^{-2}	36	5760	5151	5874	5803	3713	2681	53
10^{-1}	19	6340	6684	4699	5756	3981	3299	31
1	21	5883	6893	5177	5997	4281	3191	41
10	29	4537	6229	4610	6580	3777	2131	76
10^2	20	3822	5628	4370	4989	4129	2168	30
10^3	14	2386	3470	2081	3281	2315	941	19
10^4	33	1407	1456	1066	1083	447	189	19

Table 19c. Effect of Leukocyte A Interferon (AVS 4625) on Proliferation of Clone TFE 96.

T cell clones were incubated with irradiated autologous feeders, antigen, and the indicated concentrations (U/ml) of AVS 4625. After 48 hours, cultures were pulsed with ^{3}H-thymidine for 18 hours and incorporation of label into DNA was measured. Results are expressed as mean cpm for triplicate cultures.

[AVS 4625]	Medium	CM	Flu A 1:1000	Flu A 1:2000	Flu A 1:4000	Flu A 1:8000	Flu A 1:16000	Flu B 1:1000
0	19	6033	5180	5381	2307	1368	573	102
10^{-2}	15	5917	7355	4608	2831	1617	795	27
10^{-1}	21	5636	6610	4759	2507	1822	569	19
1	22	5405	6599	5197	2793	1287	475	17
10	24	4946	5283	3473	2919	1619	702	19
10^2	19	2943	4300	3089	1470	1043	235	20
10^3	20	2175	2561	1447	817	493	120	9
10^4	15	1562	801	276	138	54	37	16

Table 19d. Effect of Leukocyte A Interferon (AVS 4625) on Proliferation of Clone TFE 97.

T cell clones were incubated with irradiated autologous feeders, antigen, and the indicated concentrations (U/ml) of AVS 4625. After 48 hours, cultures were pulsed with ^{3}H-thymidine for 18 hours and incorporation of label into DNA was measured. Results are expressed as mean cpm for triplicate cultures.

[AVS 4625]	Medium	CM	Flu A 1:1000	Flu A 1:2000	Flu A 1:4000	Flu A 1:8000	Flu A 1:16000	Flu B 1:1000
0	31	4837	3995	3316	1888	661	191	25
10^{-2}	17	5499	5251	3279	2441	873	453	22
10^{-1}	18	4662	5216	3185	1430	1344	260	21
1	14	5382	4891	2921	1252	702	531	14
10	19	4721	4078	2865	1319	414	411	19
102	19	2948	3469	1559	803	439	44	17
10^3	23	2431	1082	783	128	64	19	8
10^4	16	1707	526	137	65	22	14	20

Table 19e. Effect of Leukocyte A Interferon (AVS 4625) on Proliferation of Clone TFE 102.

T cell clones were incubated with irradiated autologous feeders, antigen, and the indicated concentrations (U/ml) of AVS 4625. After 48 hours, cultures were pulsed with ^{3}H-thymidine for 18 hours and incorporation of label into DNA was measured. Results are expressed as mean cpm for triplicate cultures.

[AVS 4625]	Medium	CM	Flu A 1:1000	Flu A 1:2000	Flu A 1:4000	Flu A 1:8000	Flu A 1:16000	Flu B 1:1000
0	73	8537	11202	6481	3363	1029	1094	39
10^{-4}	49	8066	11747	10587	2714	1391	1300	67
10^{-3}	64	8117	12737	7863	3700	4409	997	21
10^{-2}	35	8022	12440	10557	3325	1835	1590	24
10^{-1}	29	8461	13016	10835	3431	1951	1254	33
1	47	7038	11399	10196	2843	1674	1145	43
10	21	5765	9971	10403	3035	1539	1944	40
10^2	45	4553	9717	7321	1502	1246	375	18

Table 20a. Effect of Leukocyte A Interferon (AVS 4625) on Proliferation of Clone TFE 40.

T cell clones were incubated with irradiated autologous feeders, antigen, and the indicated concentrations (U/ml) of AVS 4625. After 48 hours, cultures were pulsed with ^{3}H-thymidine for 18 hours and incorporation of label into DNA was measured. Results are expressed as mean cpm for triplicate cultures.

[AVS 4625]	Medium	CM	Flu A 1:1000	Flu A 1:2000	Flu A 1:4000	Flu A 1:8000	Flu A 1:16000	Flu B 1:1000
0	34	5407	11667	10966	5273	3345	1489	75
10^{-4}	84	6087	12758	11800	6091	2891	2004	133
10^{-3}	181	5909	12201	11499	5159	3503	2438	50
10^{-2}	247	6370	12127	11952	5503	3700	2015	39
10^{-1}	81	6129	12427	12278	5122	3554	1519	57
1	49	5340	12351	11800	4163	4013	608	12
10	18	4373	13664	10702	4017	2981	1565	17
10^2	38	3523	12705	10771	3158	1713	880	18

Table 20b. Effect of Leukocyte A Interferon (AVS 4625) on Proliferation of Clone TFE 89.

T cell clones were incubated with irradiated autologous feeders, antigen, and the indicated concentrations (U/ml) of AVS 4625. After 48 hours, cultures were pulsed with ^{3}H-thymidine for 18 hours and incorporation of label into DNA was measured. Results are expressed as mean cpm for triplicate cultures.

[AVS 4625]	Medium	CM	Flu A 1:1000	Flu A 1:2000	Flu A 1:4000	Flu A 1:8000	Flu A 1:16000	Flu B 1:1000
0	26	7037	9581	8649	2717	695	707	49
10^{-4}	18	6653	8791	9747	3789	1656	1060	27
10^{-3}	16	6305	9929	9031	4423	1346	887	18
10^{-2}	17	6109	9217	8630	3099	2875	1387	17
10^{-1}	22	6330	10131	8645	4431	2216	895	32
1	14	5981	8463	8274	4303	1753	944	31
10	55	4760	10160	8565	3382	1993	1001	13
10^2	22	3663	8930	7964	3029	586	537	17

Table 20c. Effect of Leukocyte A Interferon (AVS 4625) on Proliferation of Clone TFE 96.

T cell clones were incubated with irradiated autologous feeders, antigen, and the indicated concentrations (U/ml) of AVS 4625. After 48 hours, cultures were pulsed with ^{3}H-thymidine for 18 hours and incorporation of label into DNA was measured. Results are expressed as mean cpm for triplicate cultures.

[AVS 4625]	Medium	CM	Flu A 1:1000	Flu A 1:2000	Flu A 1:4000	Flu A 1:8000	Flu A 1:16000	Flu B 1:1000
0	21	7053	10705	8445	2795	1444	516	37
10^{-4}	19	7284	11945	9426	3803	1937	1766	23
10^{-3}	25	7293	11443	9120	5077	2565	1538	14
10^{-2}	27	6823	11881	10377	5032	2479	1671	19
10^{-1}	18	7205	12270	9515	4692	1414	1074	12
1	27	6293	11751	9999	4138	2415	573	22
10	16	5557	11344	10101	4486	1162	473	24
10^2	16	6103	8911	3247	1801	1061	343	25

Table 20d. Effect of Leukocyte A Interferon (AVS 4625) on Proliferation of Clone TFE 97.

T cell clones were incubated with irradiated autologous feeders, antigen, and the indicated concentrations (U/ml) of AVS 4625. After 48 hours, cultures were pulsed with ^{3}H-thymidine for 18 hours and incorporation of label into DNA was measured. Results are expressed as mean cpm for triplicate cultures.

[AVS 4625]	Medium	CM	Flu A 1:1000	Flu A 1:2000	Flu A 1:4000	Flu A 1:8000	Flu A 1:16000	Flu B 1:1000
0	21	6808	8283	5442	1026	328	137	29
10^{-4}	15	6623	9178	6659	1354	1010	420	19
10^{-3}	25	7043	9387	6106	1269	1076	132	25
10^{-2}	21	6526	9360	7365	1379	1161	494	21
10^{-1}	17	6212	8793	5797	2395	802	297	16
1	21	5695	7261	6180	1783	309	93	20
10	18	4791	7163	5531	1119	175	26	20
10^2	11	3877	4787	4021	731	122	19	10

Table 20e. Effect of Leukocyte A Interferon (AVS 4625) on Proliferation of Clone TFE 102.

T cell clones were incubated with irradiated autologous feeders, antigen, and the indicated concentrations (U/ml) of AVS 4625. After 48 hours, cultures were pulsed with ^{3}H-thymidine for 18 hours and incorporation of label into DNA was measured. Results are expressed as mean cpm for triplicate cultures.

[AVS 4625]	TFE 23	TFE 79	TFE 89
0	66.5	5.3	17.0
10^{-3} U/ml	59.9	4.8	17.1
10^{-2}	54.2	3.9	13.5
10^{-1}	59.2	6.6	17.3
1	69.5	15.8	30.1
10	66.2	16.7	25.4
10^{2}	67.6	19.0	25.4
10^{3}	69.6	12.7	22.7
10^{4}	66.7	16.4	24.2

Table 21. Effect of Leukocyte A Interferon (AVS 4625) on Virus-Specific Cytotoxicity.

T cell clones were incubated with the indicated concentrations of AVS 4625 for 1 hour prior to the addition of target cells. Results are expressed as % specific lysis.

[AVS 5311]	Medium	CM	Flu A 1:1000	Flu A 1:2000	Flu A 1:4000	Flu A 1:8000	Flu A 1:16,000	Flu B 1:1000
0	449	6536	6718	6581	5008	3487	3011	269
10 -2	147	6120	6491	6271	5697	4871	3097	291
10 -1	444	5215	7103	6049	5287	3685	2818	276
1	129	5262	6381	6441	4530	3725	2196	401
10	52	5055	6149	6461	4891	3817	2744	138
10 2	77	4002	6031	6290	5195	4205	2823	111
10 3	52	3379	4945	4487	3885	3031	2065	76
10 4	65	2241	2792	3157	1937	1497	895	113

Table 22a. Effect of AVS 5311 on Proliferation of Clone TFE 40

[AVS 5311]	Medium	CM	Flu A 1:1000	Flu A 1:2000	Flu A 1:4000	Flu A 1:8000	Flu A 1:16,000	Flu B 1:1000
0	81	5929	10769	10223	7073	4585	2397	253
10 -2	53	6153	9918	10205	5739	3591	3302	308
10 -1	81	5513	9433	8704	6594	4229	2727	159
1	283	5349	9192	8605	6001	3997	3127	67
10	83	4949	7843	8638	5951	4135	3486	356
10 2	47	4110	8340	8540	4580	3504	2116	138
10 3	93	3250	7301	7169	4931	3391	1717	43
10 4	51	2402	4281	4029	2515	923	473	43

Table 22b. Effect of AVS 5311 on Proliferation of Clone TFE 89.

[AVS 5311]	Medium	CM	Flu A 1:1000	Flu A 1:2000	Flu A 1:4000	Flu A 1:8000	Flu A 1:16,000	Flu B 1:1000
0	26	4034	2323	2083	1643	1867	1206	47
10 -2	27	3841	2224	2708	2255	2120	1525	373
10 -1	22	3869	2323	2278	2072	1475	907	169
1	37	3728	2105	2542	2381	1955	1511	103
10	19	3101	2112	2029	1891	1566	1249	127
10 2	31	2385	2036	2144	2015	1704	1253	149
10 3	25	2314	1599	1646	1403	1253	775	149
10 4	17	1395	859	909	943	853	409	191

Table 22c. Effect of AVS 5311 on Proliferation of Clone TFE 96

[AVS 5311]	Medium	CM	Flu A 1:1000	Flu A 1:2000	Flu A 1:4000	Flu A 1:8000	Flu A 1:16,000	Flu B 1:1000
0	19	3207	171	172	68	29	19	30
10 -2	18	2809	133	231	73	27	21	21
10 -1	23	2905	98	134	55	45	17	19
1	19	2821	209	121	64	73	38	19
10	19	2705	162	111	62	59	69	33
10 2	26	2179	157	99	147	112	50	38
10 3	21	1294	181	56	90	162	81	77
10 4	29	679	137	98	91	92	25	27

Table 22d. Effect of AVS 5311 on Proliferation of Clone TFF 97

[AVS 5311]	Medium	CM	Flu A 1:1000	Flu A 1:2000	Flu A 1:4000	Flu A 1:8000	Flu A 1:16,000	Flu B 1:1000
0	19	6051	2455	1740	928	501	164	27
10^{-2}	36	5849	2631	1651	805	487	373	31
10^{-1}	39	5476	2767	1687	1103	785	175	40
1	25	6035	2839	2079	855	490	123	29
10	29	3012	1961	1690	477	387	152	19
10^{2}	21	4183	1958	2518	721	276	129	37
10^{3}	28	3063	1791	1015	393	206	69	29
10^{4}	48	2028	863	773	229	133	24	27

Table 20e. Effect of AVS 5311 on Proliferation of Clone TFE 102

[AVS 5311]	% Specific Lysis
0	23.5
10^{-3} U/ml	22.3
10^{-2}	22.8
10^{-1}	24.7
1	24.2
10	31.2
10^{2}	33.6
10^{3}	37.2
10^{4}	32.8

Table 23. Effect of Hybrid Human Interferon Alpha BD (AVS 5311) on Virus-Specific Cytotoxicity.

Clone TFE 89 was preincubated with the indicated concentrations of AVS 5311 for 1 hour prior to the addition of Flu A coated ^{51}Cr labelled target cells.

[AVS 5311]	TFE 18	TFE 23	TFE 79	TFE 89
0	26.1	39.1	17.9	34.6
10^{-6} U/ml	34.5	34.6	22.7	41.7
10^{-5}	32.0	36.8	24.9	40.6
10^{-4}	33.9	40.3	25.9	43.0
10^{-3}	30.7	36.4	23.0	45.2
10^{-2}	25.6	44.9	19.9	47.2
10^{-1}	30.9	41.3	22.0	43.3
1	26.6	42.0	25.0	46.6
10	36.7	47.2	23.1	42.6
10^{2}	37.8	38.4	27.6	39.5
10^{3}	34.5	43.8	24.8	39.3
10^{4}	35.4	41.2	26.1	38.2
10^{5}	33.3	44.7	26.4	40.7

Table 24. Effect of Hybrid Human Interferon Alpha BD (AVS 5311) on Virus-Specific Cytotoxicity.

The indicated concentrations of AVS 5311 were added to cytotoxic effectors at the time of target cell addition. Results are expressed as % specific lysis.

[Poly ICLC]	Medium	CM	Flu A 1:1000	Flu A 1:2000	Flu A 1:4000	Flu A 1:8000	Flu A 1:16000	Flu B 1:1000
0 [a]	11[b]	6830	9299	8636	4075	3883	1935	45
10^{-5}	19	6913	11229	8102	5282	3784	1533	23
10^{-4}	23	6627	9604	9186	4901	3262	2614	11
10^{-3}	29	7383	10562	9953	5769	3055	1137	44
10^{-2}	19	6534	9600	8669	3883	1855	1097	12
10^{-1}	19	6382	9431	7128	1819	839	264	16
1	52	5879	6075	3966	414	195	24	105
10	15	5435	4949	2851	275	99	52	33

Table 25a. Effect of Poly ICLC (AVS 1761) on Proliferation of Clone TFE 40.

T cell clones were incubated with irradiated autologous feeders, antigen, and the indicated concentrations (ug/ml) of Poly ICLC. After 48 hours, cultures were pulsed with ^{3}H-thymidine for 18 hours and incorporation of label into DNA was measured. Results are expressed as mean cpm for triplicate cultures.

[Poly ICLC]	Medium	CM	Flu A 1:1000	Flu A 1:2000	Flu A 1:4000	Flu A 1:8000	Flu A 1:16000	Flu B 1:1000
0	21	4824	7017	7250	5451	3593	2252	98
10^{-5}	25	4447	7379	8715	7283	3865	2683	25
10^{-4}	18	5103	8334	8047	5711	4181	2388	12
10^{-3}	42	4738	9706	7316	6156	4751	3789	29
10^{-2}	21	4624	8011	6744	5757	3515	2437	37
10^{-1}	12	4071	7170	5871	3555	1754	796	43
1	17	4037	4873	2405	1521	655	167	25
10	22	3385	2780	2199	1073	404	71	17

Table 25b. Effect of Poly ICLC (AVS 1761) on Proliferation of Clone TFE 89.

T cell clones were incubated with irradiated autologous feeders, antigen, and the indicated concentrations (ug/ml) of Poly ICLC. After 48 hours, cultures were pulsed with ^{3}H-thymidine for 18 hours and incorporation of label into DNA was measured. Results are expressed as mean cpm for triplicate cultures.

[Poly ICLC]	Medium	CM	Flu A 1:1000	Flu A 1:2000	Flu A 1:4000	Flu A 1:8000	Flu A 1:16000	Flu B 1:1000
0	14	5054	5053	4287	3465	2067	427	13
10^{-5}	14	4745	5688	4869	4365	1819	1107	13
10^{-4}	22	4551	5889	4569	4143	3354	1409	11
10^{-3}	19	4457	5926	5059	5150	2695	1474	21
10^{-2}	15	4410	5280	3835	3556	3264	1013	21
10^{-1}	11	4329	5083	2909	2183	587	127	26
1	14	3859	1662	1239	817	200	179	19
10	10	3245	1258	1443	220	44	21	25

Table 25c. Effect of Poly ICLC (AVS 1761) on Proliferation of Clone TFE 96.

T cell clones were incubated with irradiated autologous feeders, antigen, and the indicated concentrations (ug/ml) of Poly ICLC. After 48 hours, cultures were pulsed with ^{3}H-thymidine for 18 hours and incorporation of label into DNA was measured. Results are expressed as mean cpm for triplicate cultures.

[Poly ICLC]	Medium	CM	Flu A 1:1000	Flu A 1:2000	Flu A 1:4000	Flu A 1:8000	Flu A 1:16000	Flu B 1:1000
0	11	6272	8427	7419	6760	4015	2477	22
10^{-5}	13	6125	8867	8267	6437	4399	2997	26
10^{-4}	22	7292	9626	7843	7240	4850	2369	39
10^{-3}	15	5649	9723	7407	6932	5717	2837	26
10^{-2}	19	6143	9773	7193	7071	3663	2570	55
10^{-1}	13	5873	6967	5968	4876	2745	1353	11
1	16	5201	3173	1922	1174	587	199	17
10	10	4660	1717	1011	860	593	101	13

Table 25d. Effect of Poly ICLC (AVS 1761) on Proliferation of Clone TFE 97.

T cell clones were incubated with irradiated autologous feeders, antigen, and the indicated concentrations (ug/ml) of Poly ICLC. After 48 hours, cultures were pulsed with ^{3}H-thymidine for 18 hours and incorporation of label into DNA was measured. Results are expressed as mean cpm for triplicate cultures.

[Poly ICLC]	Medium	CM	Flu A 1:1000	Flu A 1:2000	Flu A 1:4000	Flu A 1:8000	Flu A 1:16000	Flu B 1:1000
0	25	6708	6479	4937	2909	993	317	15
10^{-5}	20	6194	8025	7231	3210	1903	187	16
10^{-4}	21	6343	9145	6853	3759	1396	309	24
10^{-3}	19	6159	8702	6766	3200	1978	176	42
10^{-2}	13	6165	7855	4716	2998	604	371	21
10^{-1}	13	6315	6845	3362	1945	571	137	25
1	13	5535	3096	1255	573	90	39	17
10	13	4641	1695	868	216	129	25	13

Table 25e. Effect of Poly ICLC (AVS 1761) on Proliferation of Clone TFE 102.

T cell clones were incubated with irradiated autologous feeders, antigen, and the indicated concentrations (ug/ml) of Poly ICLC. After 48 hours, cultures were pulsed with ^{3}H-thymidine for 18 hours and incorporation of label into DNA was measured. Results are expressed as mean cpm for triplicate cultures.

[Poly ICLC]	% Specific Lysis
0	15.7
10^{-5}	26.9
10^{-4}	24.6
10^{-3}	25.6
10^{-2}	27.6
10^{-1}	27.0
1	23.6
10	24.1

Table 26. Effect of Poly ICLC (AVS 1761) on Virus-Specific Cytotoxicity.

Clone TFE 89 was preincubated with the indicated concentrations of Poly ICLC (ug/ml) for 2 hours prior to the addition of target cells. Targets were incubated with Flu A (1:250) for 24 hours prior to assay.

[AVS 2149]	Medium	CM	Flu A 1:1000	Flu A 1:2000	Flu A 1:4000	Flu A 1:8000	Flu A 1:16000	Flu B 1:1000
0	98	8033	2435	1412	753	791	377	32
10^{-6}	24	7458	3428	1849	770	1537	1021	35
10^{-5}	96	7689	2197	1950	2622	1231	525	90
10^{-4}	50	8552	3412	1564	956	718	521	82
10^{-3}	51	6960	2761	1658	763	419	931	37
10^{-2}	32	7059	2251	1497	837	410	451	35
10^{-1}	47	7987	3409	2476	1426	551	701	53
1	61	7337	3694	3194	1231	2776	1867	37

Table 27a. Effect of Poly I.Poly C 12U (AVS 2149) on Proliferation of Clone TF E40.

T cell clones were incubated with irradiated autologous feeders, antigen, and the indicated concentrations (ug/ml) of AVS 2149. After 48 hours, cultures were pulsed with ^{3}H-thymidine for 18 hours and incorporation of label into DNA was measured. Results are expressed as mean cpm for triplicate cultures.

[AVS 2149]	Medium	CM	Flu A 1:1000	Flu A 1:2000	Flu A 1:4000	Flu A 1:8000	Flu A 1:16000	Flu B 1:1000
0	39	5014	3480	2197	1517	913	627	23
10^{-6}	48	5487	3291	2215	1633	879	854	43
10^{-5}	43	5171	4093	1920	2426	1459	893	59
10^{-4}	57	5376	4516	3395	1507	1833	929	29
10^{-3}	60	5955	4113	3828	2355	1552	787	29
10^{-2}	47	5147	3972	2899	2017	1458	971	26
10^{-1}	33	5369	3520	1618	2159	1359	414	21
1	27	5001	2488	3514	3747	2731	2628	22

Table 27b. Effect of Poly I.Poly C 12U (AVS 2149) on Proliferation of Clone TF E89.

T cell clones were incubated with irradiated autologous feeders, antigen, and the indicated concentrations (ug/ml) of AVS 2149. After 48 hours, cultures were pulsed with ^{3}H-thymidine for 18 hours and incorporation of label into DNA was measured. Results are expressed as mean cpm for triplicate cultures.

[AVS 2149]	Medium	CM	Flu A 1:1000	Flu A 1:2000	Flu A 1:4000	Flu A 1:8000	Flu A 1:16000	Flu B 1:1000
0	28	5259	3801	3005	1410	889	515	25
10^{-6}	39	6297	3756	2699	1226	1108	545	62
10^{-5}	61	5821	3442	1529	2229	846	685	26
10^{-4}	23	5721	4312	2294	2257	1537	693	75
10^{-3}	27	6032	3350	2412	1822	1705	609	23
10^{-2}	50	5829	4589	2743	1627	2391	1419	35
10^{-1}	77	5866	3817	2290	1399	877	816	31
1	31	5665	3401	3233	2665	1953	1343	33

Table 27c. Effect of Poly I.Poly C 12U (AVS 2149) on Proliferation of Clone TF E96.

T cell clones were incubated with irradiated autologous feeders, antigen, and the indicated concentrations (ug/ml) of AVS 2149. After 48 hours, cultures were pulsed with ^{3}H-thymidine for 18 hours and incorporation of label into DNA was measured. Results are expressed as mean cpm for triplicate cultures.

[AVS 2149]	Medium	CM	Flu A 1:1000	Flu A 1:2000	Flu A 1:4000	Flu A 1:8000	Flu A 1:16000	Flu B 1:1000
0	103	5805	3183	1871	882	458	213	33
10^{-6}	27	5733	3068	1496	2003	577	405	36
10^{-5}	87	6005	3907	1721	1672	974	466	47
10^{-4}	86	5693	4163	2127	1147	695	353	103
10^{-3}	47	5880	4901	2367	1731	587	468	153
10^{-2}	31	5430	4006	1612	957	831	409	29
10^{-1}	35	5645	3847	1338	1304	1161	196	46
1	45	5525	2705	1469	2223	1230	754	35

Table 27d. Effect of Poly I.Poly C 12U (AVS 2149) on Proliferation of Clone TF E97.

T cell clones were incubated with irradiated autologous feeders, antigen, and the indicated concentrations (ug/ml) of AVS 2149. After 48 hours, cultures were pulsed with ^{3}H-thymidine for 18 hours and incorporation of label into DNA was measured. Results are expressed as mean cpm for triplicate cultures.

[AVS 2149]	TFE 23	TFE 79	TFE 89
0	66.5	5.3	17.0
10^{-5} ug/ml	62.1	1.8	11.2
10^{-4}	60.0	2.6	10.0
10^{-3}	60.1	4.5	15.6
10^{-2}	63.6	1.5	10.6
10^{-1}	62.6	3.4	11.2
1	60.3	0.9	8.9
10	51.4	1.3	12.3

Table 28. Effect of Poly I.Poly C 12U (AVS 2149) on Virus-Specific Cytotoxicity.

The indicated concentrations of AVS 2149 were added to cytotoxic effector at the time of target cell addition. Results are expressed as % specific lysis.

[AVS 2149]	TFE 18	TFE 23	TFE 79	TFE 89
0	47.8	78.2	15.3	48.6
10^{-5} ug/ml		62.3	20.5	43.4
10^{-4}		65.2	18.3	41.5
10^{-3}		67.9	14.2	44.2
10^{-2}		62.7	8.8	41.8
10^{-1}	32.9	64.1	18.0	43.2
1	21.7	52.8	15.3	37.3
10	27.2	48.7	14.3	35.9

Table 29. Effect of Poly I.Poly C 12U (AVS 2149) on Virus-Specific Cytotoxicity.

Effector cells were incubated with the indicated concentrations of AVS 2149 for 1 hour before addition of target cells. Results are expressed as % specific lysis.

[MTP]	Medium	CH	Flu A 1:1000	Flu A 1:2000	Flu A 1:4000	Flu A 1:8000	Flu A 1:16,000	Flu B 1:1000
0	313	8851	5298	5055	3510	2004	2099	713
10 -7	477	7819	5675	3359	3261	1943	1625	546
10 -6	420	7901	6025	4753	4361	2089	1947	552
10 -5	200	8196	6692	4726	3951	3085	1748	887
10 -4	352	8114	6345	5143	4020	2084	2890	438
10 -3	679	8604	5617	4845	4174	1759	2882	841
10 -2	451	7406	5276	4215	3949	2624	1922	543
10 -1	101	7431	5387	3836	1845	741	640	144
1	140	6643	1545	499	1260	868	280	147

Table 30a. Effect of MTP on Proliferation of Clone TFE 40

[Placebo]	Medium	CM	Flu A 1:1000	Flu A 1:2000	Flu A 1:4000	Flu A 1:8000	Flu A 1:16,000	Flu B 1:1000
0	313	8851	5298	5055	3510	2004	2099	713
10 -7	224	8291	5191	3482	2580	1311	1434	220
10 -6	166	7753	5116	3707	3698	3017	1795	329
[illegible]	544	8586	5315	3467	3525	1981	1511	249
[illegible]	181	8858	6237	4595	3069	2395	1569	107
10 -3	273	8818	[illegible]	4038	3402	1503	1148	583
[illegible]	[illegible]	[illegible]	[illegible]	3444	[illegible]	[illegible]	855	549
10 -1	193	7191	4113	2549	2150	1434	[illegible]	129
1	96	6234	1371	1340	1300	657	761	[illegible]

Table [illegible]. Effect of Placebo Liposomes on Proliferation of Clone TFE 40

[MTP]	Medium	CM	Flu A 1:1000	Flu A 1:2000	Flu A 1:4000	Flu A 1:8000	Flu A 1:16,000	Flu B 1:1000
0	282	7021	9843	8843	6251	3891	2760	840
10 -7	479	9067	10397	7327	7341	3935	2751	170
10 -6	730	8568	10007	7030	4851	3580	1987	605
10 -5	501	8965	11583	8923	6036	3629	2784	171
10 -4	771	8845	9208	9096	4603	3232	3493	649
10 -3	827	8851	10549	7432	5907	3246	1666	399
10 -2	233	8231	8967	6789	3880	3694	1555	531
10 -1	371	7986	6216	4604	2468	914	609	400
1	97	6766	4007	2879	1857	289	447	70

Table 31a. Effect of MTP on Proliferation of Clone TFE 89

Placebo	Medium	CM	Flu A 1:1000	Flu A 1:2000	Flu A 1:4000	Flu A 1:8000	Flu A 1:16,000	Flu B 1:1000
[illegible]	282	7021	9843	8843	[illegible]251	3891	2760	840
10 -7	194	8938	[illegible]4	8326	4277	2378	1632	409
10 -6	151	9089	9807	5795	46[illegible]	[illegible]36	1767	437
10 -5	693	9011	9543	5690	5379	2823	[illegible]083	315
10 -4	305	9123	10571	7295	4592	2739	1195	515
10 -3	356	9367	9183	8323	4310	2417	2363	76
10 -2	174	8197	8955	7799	4533	3943	997	217
10 -1	132	8815	6903	4742	2721	2056	1604	177
1	109	7638	5595	4791	2239	1807	854	95

Tabale 31b. Effect of Placebo Liposomes on Proliferation of Clone TFE 89.

[MTP]	Medium	CM	Flu A 1:1000	Flu A 1:2000	Flu A 1:4000	Flu A 1:8000	Flu A 1:16,000	Flu B 1:1000
0	451	8928	7748	8138	7020	6243	3033	314
10 -7	163	7960	8780	6920	7071	5105	4091	171
10 -6	65	8367	8895	7385	6050	4701	3925	175
10 -5	82	7574	9777	7989	7375	5715	3613	467
10 -4	61	7929	8749	7199	6364	6185	3611	325
10 -3	322	7309	8647	7167	6248	4883	2387	465
10 -2	119	8215	10601	5967	5403	3891	2529	253
10 -1	73	8138	4361	5163	4908	2813	1369	139
1	181	7107	6327	4128	3548	2432	1031	52

Table 32a. Effect of MTP on Proliferation of Clone TFE 96

Placebo	Medium	CM	Flu A 1:1000	Flu A 1:2000	Flu A 1:4000	Flu A 1:8000	Flu A 1:16,000	Flu B 1:1000
0	413	8849	7767	8111	7145	6304	3081	299
10 -7	63	9505	8427	6052	5957	4555	3785	207
10 -6	266	9602	11653	7503	7264	4376	3313	257
10 -5	312	8963	7915	7593	7465	5855	2937	327
10 -4	229	8867	8230	7474	6689	5301	3571	313
10 -3	397	8728	9510	8346	6372	6156	2575	317
10 -2	167	9173	8967	7241	5909	5029	1963	118
10 -1	67	8615	8390	6877	4249	3781	2053	200
1	45	8237	6688	5275	4975	3899	1880	287

Table 32b. Effect of Placebo Liposomes on Proliferation of Clone TFE 96

[MTP]	Medium	CM	Flu A 1:1000	Flu A 1:2000	Flu A 1:4000	Flu A 1:8000	Flu A 1:16,000	Flu B 1:1000
0	332	6663	10960	10244	6778	6821	2699	1177
10 -7	362	8142	8765	6671	6077	4625	2812	532
10 -6	492	8154	9957	6999	6283	3879	3098	535
10 -5	287	8020	9745	7935	6279	4139	1843	431
10 -4	299	8289	9396	7010	6259	4423	1923	626
10 -3	397	8549	9119	7345	4731	3027	1745	369
10 -2	294	8206	8653	5303	4095	3485	1522	233
10 -1	166	7713	7635	6877	3910	2040	776	315
1	89	7861	5441	3697	2874	1890	656	231

Table 33a. Effect of MTP on Proliferation of Clone TFE 97

[Placebo]	Medium	CM	Flu A 1:1000	Flu A 1:2000	Flu A 1:4000	Flu A 1:8000	Flu A 1:16,000	Flu B 1:1000
0	349	6738	11001	9967	6795	6901	2679	1150
10 -7	148	9264	10348	8065	5996	4242	2187	345
10 -6	184	9383	8928	8663	7435	5884	2403	412
10 -5	279	8265	11559	7676	7690	5321	1655	118
10 -4	147	10107	9529	8079	6679	3930	2584	509
10 -3	221	9167	10466	7487	5727	3662	2294	457
10 -2	287	8937	10619	6941	4763	2648	803	328
10 -1	72	9216	10059	6531	4707	2251	1881	138
1	213	8743	7723	4953	4281	2141	1506	362

Table 35b. Effect of Placebo Liposomes on Proliferation of Clone TFE 97

[MTP]	Medium	CM	Flu A 1:1000	Flu A 1:2000	Flu A 1:4000	Flu A 1:8000	Flu A 1:16,000	Flu B 1:1000
0	279	6379	7042	5881	3872	2581	817	553
10 -7	107	8081	5585	4201	2701	1973	1220	373
10 -6	247	7621	6379	3931	3997	2532	1284	481
10 -5	91	7368	5054	4464	3305	2594	1374	137
10 -4	105	7915	5695	3967	2949	1176	1097	132
10 -3	199	7181	5641	4223	3194	1783	483	150
10 -2	86	7833	5853	3465	3350	1851	441	369
10 -1	49	7173	4229	3360	2213	1279	397	80
1	115	7093	2667	1637	1337	709	156	176

Table 34a. Effect of MTP on Proliferation of Clone TFE 102

[Placebo]	Medium	CM	Flu A 1:1000	Flu A 1:2000	Flu A 1:4000	Flu A 1:8000	Flu A 1:16,000	Flu B 1:1000
0	275	6405	6919	5961	3988	2582	765	541
10 -7	143	7771	5141	5459	2845	1689	517	61
10 -6	57	8685	6551	4515	2983	1630	676	268
10 -5	75	8881	6094	5599	3391	1955	1023	73
10 -4	169	9003	6168	4643	3277	1955	751	137
10 -3	46	8181	6669	3831	2685	2067	804	331
10 -2	49	8827	5215	4117	2353	1796	191	150
10 -1	52	9216	5683	3935	1429	509	425	325
1	69	8048	4306	2226	2313	1593	305	97

Table 34b. Effect of Placebo Liposomes on Proliferation of Clone TFE 102

	% Specific Lysis	
	MTP	Placebo
0	23.5	23.5
10^{-7} ug/ml	20.5	18.8
10^{-6}	22.8	20.4
10^{-5}	19.8	19.3
10^{-4}	23.3	20.5
10^{-3}	21.3	20.7
10^{-2}	21.5	20.4
10^{-1}	23.2	21.0
1	21.1	17.9

Table 35. Effect of MTP on Virus-Specific Cytotoxicity.

Clone TFE 89 was preincubated with the indicated concentrations of MTP (AVS 2933) or placebo liposomes (AVS 4726) for 1 hour prior to the addition of Flu A coated ^{51}Cr labelled target cells.

Clone	[AVS 1018]	Medium	Flu 1:8000	Flu 1:16,000
TFE89	0	104	2077	
	10^{-5}	64	2783	
TFE96	0	65	531	396
	10^{-5}	31	1339	622
	10^{-4}	36	967	720
TFE97	0	69	1852	1256
	10^{-5}	72	3112	
	10^{-3}	122		2583

Table 36. Effect of AVS 1018 on T Cell Clonal Proliferation.

No significant effect on antigen-specific proliferation of clones TFE40 or TFE102 was seen at any concentration of AVS 1018.

Clone	[AVS 1300]	Medium	Flu 1:8000	Flu 1:16,000
TFE40	0	99	5613	
	10^{-3}	60	10,793	
TFE89	0	88	7883	6685
	10^{-5}	65	12,384	8394
	10^{-4}	49	9257	8681
	10^{-1}	117	9644	10,746
TFE96	0	97	5627	3560
	10^{-3}	50	7743	6356
TFE97	0	221	5076	3248
	10^{-5}	67	8761	5583
	10^{-1}	77	9306	4645
TFE102	0	981	10,083	5901
	10^{-6}	263	14,713	9437
	10^{-2}	224	12,617	9899

Table 37. Effect of AVS 1300 on T Cell Clonal Proliferation.

[AVS 1018]	TFE 18	TFE 23	TFE 79	TFE 89
0	63.8	46.4	3.6	14.6
10^{-7} ug/ml	60.3	33.0	4.9	10.3
10^{-6}	59.0	23.0	2.3	7.8
10^{-5}	58.1	27.4	2.1	10.8
10^{-4}	51.8	33.4	1.2	8.8
10^{-3}	69.4	34.3	3.9	10.6
10^{-2}	57.3	37.6	3.5	11.3
10^{-1}	61.3	36.2	1.2	16.1
1	48.9	33.4	8.8	11.1

Table 38. Effect of AVS 1018 on Virus-Specific Cytotoxicity.

Effector cells were incubated with the indicated concentrations of AVS 1018 for 1 hour before addition of target cells. Results are expressed as % specific lysis.

[AVS 1300]	TFE 18	TFE 23	TFE 79	TFE 89
0	63.8	46.4	3.6	14.6
10^{-7} ug/ml	35.8	30.8	0.2	8.1
10^{-6}	47.6	27.3	-4.3	9.0
10^{-5}	41.2	39.7	0.8	20.6
10^{-4}	44.4	41.0	-4.9	19.9
10^{-3}	51.6	52.5	-1.0	10.9
10^{-2}	49.0	52.7	-1.2	13.0
10^{-1}	37.5	51.8	-1.4	9.9
1	46.4	45.1	-0.8	14.3

Table 39. Effect of AVS 1300 on Virus-Specific Cytotoxicity.

Effector cells were incubated with the indicated concentrations of AVS 1300 for 1 hour before addition of target cells. Results are expressed as % specific lysis.

Clone	[AVS 2880]	Medium	Flu 1:8000	Flu 1;16,000
TFE96	0	40	3209	1687
	10^{-4}	85	4387	2300
	10^{-3}	18	4352	2017
TFE97	0	240	3213	1153
	10^{-5}	167	4747	2118
	10^{-4}	151	3504	2611
TFE102	0	518	3447	1817
	10^{-6}	156	6341	2434
	10^{-5}	74	4288	3119

Table 40. Effect of AVS 2880 on T Cell Clonal Proliferation.

No significant effect was seen on proliferation of TFE40 or TFE89.

Clone	[AVS 2776]	Medium	Flu 1:8000	Flu 1:16,000
TFE89	0	56	811	
	10^{-4}	31	2,435	
	10^{-3}	18	1,589	
TFE96	0	27	1,149	
	10^{-2}	29	1,961	
TFE97	0	15		378
	10^{-6}	34		792
	10^{-4}	18		639
TFE102	0	36	606	67
	10^{-6}	59	1,759	277
	10^{-5}	90	1,272	581
	10^{-1}	30		993

Table 41. Effect of AVS 2776 on T Cell Clonal Proliferation.

Clone	[AVS 2777]	Medium	Flu 1:2000	Flu 1:4000	Flu 1:16000
TFE40	0	37	2,743		
	10^{-3}	33	4,065		
TFE89	0	23	3,281	2,096	
	10^{-3}	23		3,168	
	1	41	5,266		
TFE96	0	35		831	155
	10^{-6}	33			599
	10^{-5}	19		1,583	411
TFE97	0	23		1,167	157
	10^{-6}	25			636
	10^{-3}	17		1,703	
TFE102	0	19		381	
	10^{-5}	19		1,165	

Table 42. Effect of AVS 2777 on T Cell Clonal Proliferation.

Clone	[AVS 2778]	Medium	Flu 1:1000	Flu 1:2000	Flu 1:16000
TFE96	0	38		2,679	
	10^{-6}	216		4,157	
TFE97	0	383	8,580		
	10^{-4}	269	11,490		
TFE102	0	483	11,875		567
	10^{-3}	159	15,602		1,893

Table 43. Effect of AVS 2778 on T Cell Clonal Proliferation.

Clone	[AVS 3587]	Medium	Flu 1:4000	Flu 1:8000	Flu 1:16000
TFE40	0	131		1,861	563
	10^{-4}	144			1,145
	10^{-1}	39		2,943	
TFE89	0	64			779
	10^{-4}	21			1,491
TFE96	0	53			259
	10^{-3}	131			593
TFE102	0	73	1,922	487	
	10^{-3}	151	3,523		
	10^{-2}	312		1,084	

Table 44. Effect of AVS 3587 on T Cell Clonal Proliferation.

APPENDIX II

Scientific Reviewer's Comments

SCIENTIFIC REVIEWER'S COMMENTS
FINAL REPORT, JULY 1, 1991
CONTRACT NO. DAMD17-86-C-6154
P.I. - MAJORIE L. COHN, Ph.D.

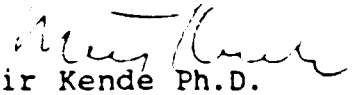
Subject: Review of Final Report Contract DAMD17-86-C-6154.

1. Interesting data was generated on the in vitro biological activity of immunomodulators, most of them have marked antiviral efficacy. In this study, influenza virus specific human T cell clones were used to assess some of the biological activity of the compounds. The lymphoproliferative response of T cell clones to various influenza virus strains was not uniform, which indicated, that these clones recognized determinants on either the hemagglutinin or neuraminidase components of the virus. Likewise, the response of the clones to the same immunomodulator was not uniform, which could mean, that different signals are required by various determinant-expressing T cells. Although, the P.I. documented the lymphoproliferative response evoked by the immunomodulators with each of the various clones, it is very difficult to draw conclusion from more than 40 tables. A summary chart would help to sort out if indeed various signals are required by the clones expressing different antigenic determinants of the influenza virus.

2. The P.I.'s claim, that certain immunomodulators inhibit the cytolysis of EB virus-shedding T cell clone, requires a set of controls which, has not been done according to the methodology provided in the report. Incubation of the target cells with test concentrations of the substances in the absence of the attacker cells would show, if the decrease in cytotoxicity is due to inhibition, or, to toxicity. The fact, that the decrease in cytotoxicity was always obtained only by high dose(s), makes the toxicity the most likely reason. The P.I. should provide the data, if she has them, to exclude toxicity.

3. A summary chart for the stimulation or depression of T cell-mediated cytotoxicity by the immunomodulators, would help to find the kind of signaling required for evoking the effector mechanism. Cytokines stimulated by most of the tested substances are known, and documented in the literature, or I can provide most of them.

4. The contract generated useful information regarding the biological responses stimulated by selected immunomodulators, but more can be learn with a little more input. The final draft should contain the requested revisions.



Meir Kende Ph.D.
COR.
Virology Division.

Note: These changes/suggestions were not made by the Principal Investigator.